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ON THE FERMENTS CONTAINED IN THE JUICE OF THE PINEAPPLE (ANANASSA SATIVA), TOGETHER WITH SOME OBSERVATIONS ON THE COMPOSITION AND PROTEOLYTIC ACTION OF THE JUICE.

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Compliments of
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[From the Transactions of the Connecticut Academy, Vol. VIII, 1891.]

On the Ferments contained in the Juice of the Pineapple (*Ananassa sativa*), together with some Observations on the Composition and Proteolytic action of the Juice.

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Some time ago the writer's attention was called to the fact that Señor V. Marcano,* of Venezuela, had discovered the existence of a proteid-digesting principle in plants of the order Bromeliaceæ, of which the pineapple is a well known representative, and that the juice of the latter fruit was being made use of as a digestive agent in the preparation of pre-digested foods.† So far as the writer is aware, there is no scientific record of this discovery other than in a short note contained in a recent number of a pharmaceutical journal,‡ in which attention is simply called to Marcano's discovery and the name "*bromelin*" suggested as an appropriate title for the hypothetical ferment.§ Apparently, no study has been made of the nature of the ferment presumably present in the juice, its mode of action, or the character of the products resulting from such action.

From a physiological standpoint the discovery of any ferment, either in the vegetable or animal kingdom, is a matter of considerable importance, especially so in the plant kingdom, since the feeling is widely gaining ground that proteid-splitting ferments must play an important part in rendering the food material of plants available. As in the animal kingdom, proteid food to be available for the needs of the plant must be transformed into soluble forms fitted for absorption and circulation. Hitherto, the best known illustration of such a vegetable proteolytic ferment has been *papaïn*, present in the juice of the papaw plant, but in the discovery of the proteolytic action of pineapple juice we have what promises to be an equally prominent illustration and one, moreover, which constitutes an addi-

* Recently deceased.

† By the Mosquera-Julia Food Co.

‡ Bulletin of Pharmacy, vol. v, p. 77, 1891.

§ Since the above was written the writer's attention has been called to the following reference contained in the Botanisches Centralblatt, No. 44, 1891: "E. Kayser, Note sur les ferments de L'ananas, Annales de l'Institute Pasteur, 1891, No. 7." To how great an extent this communication treats of the work about to be described the writer has at present no means of judging, as the above periodical is not at hand.

tional reason for believing in the probable wide-spread distribution of proteid-digesting principles throughout the vegetable kingdom.

The proteid-digesting power* of fresh pineapple juice is something quite remarkable in its intensity; it is moreover a constant feature and one which admits of easy demonstration. During the past few months great numbers of ripe pineapples have been examined in the writer's laboratory and in no instance has the juice failed to show marked proteolytic power, as evidenced by its ready solvent action on blood fibrin and other forms of proteid matter.

In addition to this proteid-digesting power, we have discovered that the juice also possesses in a remarkable degree the power of curdling or clotting milk. Neutralized pineapple juice added to milk warmed at 40° C., quickly brings about a separation of the casein, in the form of a thick clot or curd, the action being apparently exactly analogous to that of the rennet-ferment or rennin. Boiling the neutralized juice prior to its addition to the milk prevents this separation of a clot, and hence the action in question must be due to the presence of a rennet-like ferment. This ferment, indeed, we have been able to separate from the juice, together with the proteolytic ferment, by saturation of the fluid with ammonium sulphate and with this preparation we have substantiated its milk-curdling properties.

General character of pineapple juice.

As is well known, the pineapple is an exceedingly juicy fruit, an average sized one of 1100 grams yielding, after chopping the tissue and subjecting it to sufficient pressure, 600–800 cubic centimeters, or considerably more than half its weight, of juice. As it flows from the press the fluid has a somewhat turbid appearance, not easily removed by filtration through paper, but eventually as the pores of the paper become somewhat filled up a perfectly clear yellowish colored filtrate is obtained, of very decided acid reaction and with an average specific gravity of 1043. The acidity is very pronounced, but naturally quite variable, being dependent in part upon the ripeness of the fruit. A determination of the acidity of twenty distinct samples of filtered juice showed an average acidity equivalent to 0·45 per cent. hydrochloric acid (HCl), the extremes being 0·28 per cent. and 0·65 per cent., calculated as HCl. The content of proteid matter in the clear filtered juice is quite small. Heated with Millon's reagent, a fairly strong proteid reaction is

* This was referred to by the writer in a paper read before the Philadelphia County Medical Society, May 13, 1891, an abstract of which was published in the Medical News, vol. lviii, p. 719.

obtained, the intensity of which, however, appears to be due in part to the presence of tyrosin or some related soluble body.

With acetic acid alone no precipitate is produced, but with acetic acid and potassium ferrocyanide a slight precipitate or turbidity results. With the biuret test, the reaction is in great part vitiated by the large amount of sugar present in the juice, which gives rise to an intense yellowish-brown color on addition of the strong alkali.

Neutralization of the acid juice with sodium carbonate fails to give any neutralization precipitate whatever, thus showing the absence of acid-albumin. Concentrated nitric acid produces in the clear filtered juice a white precipitate soluble in excess of the acid, the fluid taking on a bright yellow color.

Fresh pineapple juice filtered clear and with average acidity, subjected to fractional heat precipitation grows slightly turbid at 60–62° C., the turbidity increasing gradually as the temperature is raised until 75–78° C. is reached, when a small flocky coagulum results. The filtrate from this coagulum on being further heated shows signs of turbidity at about 82° C., increasing with the rise in temperature, without however any distinct signs of flocking until the boiling point is reached. As the fluid commences to boil, but sometimes only after persistent boiling, a fine flocky precipitate separates, which on filtration leaves a perfectly clear fluid. This fluid, free from all matter coagulable by heat, gives with Millon's reagent the usual proteid reaction, while with acetic acid and potassium ferrocyanide, it yields a distinct white precipitate. Concentrated nitric acid alone gives no reaction, but addition of saturated-salt solution with the acid causes a distinct turbidity, which is increased rather than diminished by heat. We have thus evidence of the presence in pineapple juice of what appears to be three distinct proteids ; two separable from the acid juice by heat alone, one at about 75° C., the other at 100° C., while the third body is wholly non-coagulable by heat, but is precipitable by acetic acid and potassium ferrocyanide. This latter proteid can also be precipitated by saturation of its solution with ammonium sulphate (after removal of the proteids separable by boiling) together with some non-proteid matter present in the juice, and after removal of the ammonium sulphate by dialysis gives the reactions above indicated. It is present only in very small quantity. In some cases, however, this third body is present in the juice in larger quantity, or to express it more exactly, in some specimens the precipitate produced by acetic acid and potassium ferrocyanide in the filtrate from the heat precipitations is considerably more pronounced than first described.

The two precipitates produced by heat alone, viz: at 75–78° C. and at 100° C. are not coagulated proteids in the ordinary sense of the term. Unlike an ordinary coagulum of albumin or globulin, these precipitates, when filtered off and washed with water, dissolve readily and almost entirely in dilute solutions of potassium hydroxide; they are also more or less completely soluble in 0·5 per cent. solution of sodium carbonate, especially if warmed, and are somewhat soluble in 0·2 per cent. hydrochloric acid. In strong nitric acid on the other hand, both precipitates are insoluble unless a large quantity of the acid is added. They are likewise insoluble in 10 per cent. solution of sodium chloride. The proteid separable by heat at about 75° C. appears somewhat more soluble in 0·5 per cent. sodium carbonate than the substance separating at 100° C., the latter dissolving completely in 0·5 per cent. sodium carbonate only when the mixture is heated to boiling. The solution of the above precipitates in dilute alkali is not alkali-albumin, although the substance is in part precipitated by neutralization of the alkaline fluid, since nitric acid gives a precipitate apparently wholly insoluble in excess of the acid, even on heating.

These two proteids, when dissolved in potassium hydroxide and tested with a few drops of a dilute solution of cupric sulphate, give a violet rather than a red biuret reaction.

While thus these two proteids precipitated by heat, at 75° and 100° C. respectively, are not exactly akin to an ordinary albumin or globulin in their behavior towards heat, neither do they closely resemble on the other hand an ordinary albumose, like the β phytalbumose of Martin* present in papaw juice, since the precipitates are not readily soluble in nitric acid, even when heated, and when once dissolved are not separable on cooling the solution.

As already stated, the amount of proteid matter in pineapple juice is comparatively small and apparently these two proteids, separable by boiling, compose the greater part of the albuminous matter. The amount was determined by simply heating 100^{cc} of filtered (acid) juice to boiling, collecting the precipitate on a weighed filter, washing it thoroughly with boiling water and drying it at 110° C. until of constant weight. The result showed the presence of only 0·0270 gram in the 100^{cc} of juice, or less than 0·025 per cent.

When *neutralized* pineapple juice is subjected to heat precipitation, the initial turbidity makes its appearance at about 72°–74° C. with separation of flocks at 82°–83° C. The filtrate from this pre-

* Journal of Physiology, vol. vi, p. 347.

cipitate remains clear even when the solution is boiled, but a drop or two of acetic acid added to the hot fluid produces a turbidity, which on further heating eventually changes to a flocculent precipitate. In the filtrate from this second precipitate, acetic acid and potassium ferrocyanide show the presence, in small quantity, of what is presumably a non-coagulable proteose. It is thus evident that the presence of acid lowers the temperature at which these bodies are precipitated by heat, and further that the substance precipitated at 100° C. can be made to separate only in the presence of dilute acid. This fact certainly favors the view that the juice contains two distinct proteids precipitable by heat, the one at about 75° C. in an acid solution or 82° C. in a neutral fluid, the other at 100° C. in an acid fluid. On the other hand, the above reactions might be produced by a single substance slowly or incompletely precipitated by heat, analogous to the separation of Martin's* β phytalbumose, which is described as separating in two distinct stages, viz: at from 78°–82° C., and from 83°–95° C. As against this latter view, however, we have the apparent fact that in the pineapple juice, one of the proteids is precipitated from a neutral or acid fluid by heat, the other only from an acid fluid.

Long continued dialysis (10–12 days) of neutralized pineapple juice in running water, protected from putrefaction by addition of thymol, gives little or no separation of any proteid matter. A faint turbidity may appear, but no separation sufficiently large to collect on a filter. Such turbidity as does make its appearance clears up on the addition of a few drops of a strong solution of sodium chloride, or of dilute nitric acid; two reactions equally characteristic of a globulin or of heteroproteose.

Saturation of neutralized, or acid, pineapple juice with pure ammonium sulphate precipitates all of the proteids present in the fluid, together with a small amount of non-albuminous matter. In this precipitate are contained both the proteolytic and rennet-like ferments.

Saturation of neutralized pineapple juice with sodium chloride gives rise to a small flocculent precipitate of proteid matter, which is not at all increased by the addition of acetic acid to the salt-saturated fluid. Addition of ammonium sulphate in substance to the filtrate from the salt-saturation precipitate produces a further precipitate of proteid matter, thus suggesting a possible separation of two distinct bodies. Further, simple boiling of the filtrate from

* Journal of Physiology, vol. vi, p. 349.

the sodium chloride saturation produces a flocky precipitate, substantiating this view.

Saturation of the neutralized juice with magnesium sulphate likewise produces a precipitate of proteid matter, somewhat heavier than that induced by saturation with sodium chloride. Addition of crystals of sodium sulphate to the filtrate from the above precipitates causes finally a slight additional separation of flocculent matter containing a little proteid.

Both the sodium chloride precipitate and the magnesium sulphate precipitate are strongly proteolytic.

The general character of these precipitates will be discussed more in detail later on, in connection with the isolation of the proteolytic ferment.

Proteolytic action of fresh pineapple juice under varying conditions.

As already stated, fresh pineapple juice is strongly proteolytic, and its proteid-digesting power is manifested in a neutral, acid, or even alkaline-reacting fluid. In this respect, therefore, the ferment resembles trypsin rather than pepsin.

When blood fibrin is warmed at 40° C., or thereabout, with filtered pineapple juice of average acidity the fibrin swells up somewhat in the acid fluid, then quickly becomes disintegrated and in part dissolved, the initial action certainly being as vigorous as that of a moderately strong solution of pepsin-hydrochloric acid. There invariably remains, however, even after long-continued warming at 40° C., a fairly large insoluble residue, not of unaltered fibrin, but of finely divided antialbumid-like matter more or less soluble in weak alkaline fluids, from which it is reprecipitated by the addition of dilute acid.

Pineapple juice neutralized, or made very faintly alkaline, with dilute sodium carbonate acts apparently in much the same manner as the acid juice, except that in the alkaline-reacting fluid there is less residue of antialbumid-like matter and in the neutralized juice, naturally, an utter absence of any swelling of the fibrin. An examination of the several digestive mixtures, however, shows that the products formed in a neutral or alkaline solution are different somewhat from those formed in an acid-reacting fluid, a point which will be referred to again later on.

One of the most noticeable features in the digestive action of the pineapple ferment is its peculiar softening and disintegration of the proteid matter. This is most noticeable in a neutral solution; thus

when blood fibrin, for example, is warmed with neutralized pineapple juice, or better, with a neutral solution of the isolated ferment there is at first no sign of any digestive action whatever, but on stirring or shaking the mixture, after a sufficient length of time, the fibrin falls to pieces completely disintegrated with the production of a more or less turbid fluid, after which its solution is fairly rapid, although there invariably remains considerable insoluble matter, the same as in an acid mixture. While this action of the ferment resembles somewhat that of trypsin, there is never seen that peculiar eating into the fibrin, so characteristic of the latter ferment; the fibrin never has the appearance of being full of tiny holes, as if bored by a host of worms, so often seen in a trypsin digestion. The pineapple ferment appears simply to soften the fibrin with more or less solvent action at the same time, so that when stirred or pressed it breaks apart into larger or smaller pieces, these in turn undergoing a like change until the fibrin is thoroughly disintegrated and the soluble portion dissolved.

1.—*Influence of the reaction of the fluid.*

As is well known, trypsin* and papaïn† act best in an alkaline medium; the pineapple ferment on the other hand acts most energetically in a neutral solution, although the ferment is decidedly active in the presence of both acids and alkali carbonate.

In studying the effect of changes in the reaction of pineapple juice on its proteolytic power, or in measuring the proteolytic action of the ferment under varying conditions, the following method was, as a rule, made use of; a given volume of filtered pineapple juice, usually 100 c. c., was warmed at 40° C. for a given length of time with 10 grams of moist, freshly coagulated egg-albumin, which had been completely freed from all soluble matter by thorough washing with hot water. When the period of digestion was completed the undissolved matter was collected on a weighed filter, washed with water until all soluble bodies were removed and then dried at 110° C. until of constant weight. By subtracting the weight of the insoluble residue so obtained from the weight of dry albumin‡ equivalent to the moist albumin used in the experiment, the amount of proteid matter digested, or rather converted into soluble products, was ascertained. Obviously, however, the so-called undissolved portion of

* Studies from the Laboratory of Physiological Chem., Yale University, vol. i, p. 135.

† Martin, Journal of Physiology, vol. v, p. 221; vol. vi, p. 336.

‡ Determined by simply drying 10 grams of the sampled coagulated albumin at 110° C until of constant weight.

the albumin was in part composed of insoluble antialbumid-like matter, especially when the digestions were carried on in acid or neutral media. The method, however, afforded a fairly accurate means of measuring the proteolytic power of the ferment, as contained in pineapple juice, while the greater resistance of coagulated albumin as compared with blood fibrin seemed to offer advantages in the way of accuracy.

Experiment I.—The pineapple juice employed had an acidity equal to 0·445 per cent. HCl,* requiring 13 c. c. of a 5·0 per cent. solution of Na_2CO_3 to neutralize 100 c. c. The 10 grams of moist coagulum used in each digestive mixture contained 1·3633 grams of dry albumin (at 110° C.). The mixtures were warmed at 40° C. for 3½ hours.

	Pineapple juice.	Reaction.	Undissolved albumin.	Per cent. digested.
A	100 c. c.†	natural acidity	0·8932 gram	34·5
B	100	neutralized	0·8187	40·0

Experiment II.—The acidity of the pineapple juice employed was equal to 0·507 per cent. HCl. Necessary to neutralize 100 c. c., 14·7 c. c. of a 5·0 per cent. solution of Na_2CO_3. Weight of dry albumin equivalent to the 10 grams of moist coagulum used in each mixture, 1·3302 grams. The digestions were carried on at 40° C. for 2 hours.

	Pineapple juice.	Reaction.	Undissolved albumin.	Per cent. digested.
A	100 c. c.	natural acidity	1·0536 grams	20·8
B	100	neutralized	0·9355	29·7

Experiment III.—The acidity of the pineapple juice was not accurately determined. 15 c. c. of a dilute solution of sodium carbonate were required to neutralize 100 c. c. of juice. The weight of dry albumin contained in the 10 grams of moist coagulum used in the digestions was 1·4583 grams. The mixtures were warmed at 40° C. for 5½ hours.

	Pineapple juice.	Reaction.	Undissolved albumin.	Per cent. digested.
A	100 c. c.	natural acidity	0·8926 gram	38·8
B	100	half-neutralized	0·8733	40·2
C	100	neutralized	0·8115	44·4

From these results, it is seen that full 40 per cent. or more of coagulated egg-albumin can be converted into soluble products by the pineapple ferment, under the conditions of the above experi-

* Determined by titration with a standard solution of ammonium hydroxide.

† Plus the amount of sodium carbonate solution required for neutralization in B, and of water to make an equal dilution in A.

ments, and further that the neutralized juice is considerably more active than the unneutralized fluid. Apparently, the proteolytic action of the ferment increases with the decrease in acidity, until the neutral point is reached.

With blood fibrin, on the other hand, juice of the natural acidity appears to have a greater digestive power than the neutralized fluid, although on this point we have only a single experiment to offer. This may perhaps be explained simply by the swelling of the fibrin in the dilute organic acid, this condition possibly facilitating the action of the ferment.

Experiment IV.—The acidity of the pineapple juice was equal to 0·525 per cent. HCl. Necessary to neutralize 100 c. c. of filtered juice, 15·2 c. c. 5·0 per cent. solution of Na_2CO_3. Weight of dry albumin contained in 10 grams of moist coagulum, 1·4486 grams. Weight of dry fibrin (at 110° C.) contained in 6 grams of washed blood fibrin,* the amount used in the digestions, 2·5273 grams. The digestive mixtures were warmed at 40° C. for 2 hours.

	Pineapple juice.	Reaction.	Undissolved proteid.	Per cent. digested.
Blood fibrin . . .	100 c. c.	natural acidity	1·2435 grams	50·8
	100	neutralized	1·4321	43·3
Egg-albumin . .	100	natural acidity	1·0325	28·8
	100	neutralized	1·0096	30·3

The most noticeable feature in this experiment, aside from the point already mentioned, is the far greater digestibility of blood fibrin as compared with egg-albumin, a fact which might naturally be expected, since the same is true in the case of other well-known proteolytic ferments.

The proteids of muscle tissue are likewise more readily digested by pineapple juice than coagulated egg-albumin, full 60 per cent. of the former proteids being converted into soluble products during one hour's warming at 40° C. This is plainly shown in the following experiment, in which also the muscle proteids, like egg-albumin, are seen to be more rapidly digested in the neutralized juice than in the acid fluid.

Experiment V.—The acidity of the pineapple juice employed was equal to 0·507 per cent. HCl. Necessary to neutralize 100 c. c. of juice, 14·7 c. c. of a 5 per cent. solution of Na_2CO_3. Weight of dry

* Washed with water and salt solution, then boiled in water, alcohol, and lastly in water.

proteids contained in 10 grams of prepared muscle* tissue, 2·7258 grams.

	Pineapple juice.	Reaction.	Undissolved proteid.	Per cent. digested.
½ hour at 40° C.	100 c. c.	natural acidity	1·8945 grams	30·6
	100	neutralized	1·1366	58·4
1 hour at 40° C.	100	natural acidity	1·6368	40·1
	100	neutralized	1·0145	62·8

The acidity of pineapple juice is due to organic acids and acid salts, far weaker in their action on ferments than mineral acids. Addition of dilute mineral acid in small quantity to pineapple juice of natural acidity checks, but does not prevent the digestive action of the ferment. Thus, the addition of an equal volume of 0·2 per cent. hydrochloric acid to pineapple juice diminishes very greatly its digestive power, but does not prevent it altogether. From this we may conclude that pineapple juice can exert its proteolytic power, to a certain extent, when taken into the stomach and mixed with the acid of the gastric juice. Obviously, the addition of an equal volume of 0·2 per cent. hydrochloric acid to neutralized pineapple juice does not necessarily mean the presence of 0·1 per cent. HCl, since the acid may be in great part used up in decomposing the various salts present, and in combining with the various forms of organic matter contained in the juice.

Experiment VI.—The acidity of the pineapple juice employed was equal to 0·288 per cent. HCl. The 10 grams of moist albumin coagulum used in the digestions contained 1·7972 grams of dry albumin. The mixtures were warmed at 40° C. for 17 hours.

	Pineapple juice of natural acidity.			Undissolved albumin.	Per cent. digested.
A	100 c. c.	+100 c. c.	H_2O	1·2238 grams	32·0
B	100	+100	0·2 % HCl	1·4673	19·0

Were it not for the large quantities of salts, etc., in pineapple juice the above inhibitory action of the hydrochloric acid on the ferment would be far more pronounced. This is shown by the two following experiments :

By adding about five volumes of 95 per cent. alcohol to pineapple juice, a flocculent precipitate results composed of the proteids of the juice, together with the proteolytic ferment and some salts. On dissolving this precipitate in water a solution is obtained with marked proteolytic power.

* Prepared by soaking chopped muscle tissue, freed from fat and tendons, in water until all blood and soluble extractives were removed.

Experiment VII.—Aqueous solution of the above described alcoholic precipitate. The 10 grams of moist albumin coagulum used in the digestions contained 1·4138 grams of dry albumin. The mixtures were warmed at 40° C. for 4 hours.

	Ferment solution.	Reaction.	Undissolved albumin.	Per cent. digested.
A	$100^{cc} + 100^{cc}$ H_2O	neutral	1·0881 grams	23·1
B	100 +100 0·2 % HCl	0·1 % HCl	1·4099	0·3

Thus, with this very impure preparation of the ferment the presence of 0·1 per cent. hydrochloric acid was sufficient to entirely prevent any digestive action whatever. Doubtless, even smaller amounts of acid would have the same influence on the more perfectly isolated ferment.

As already stated, saturation of pineapple juice, either neutralized or of natural acidity, with ammonium sulphate precipitates all or nearly all of the proteids present in the fluid, the precipitate showing by its proteolytic action that it contains the ferment as well. An aqueous solution of such a precipitate, dialyzed to free it from ammonium sulphate, has a marked digestive action, but when mixed with hydrochloric acid its proteolytic power, like that of the alcohol precipitate, is immediately checked.

Experiment VIII.—Aqueous, dialyzed solution of the above described ammonium sulphate precipitate. The amount of dry albumin equivalent to the 10 grams of moist coagulum used in the digestions was 1·5120 grams. The mixtures were warmed at 40° C. for 5 hours.

	Ferment solution.	Reaction.	Undissolved albumin.	Per cent. digested.
A	$100^{cc} + 100^{cc}$ H_2O	neutral	1·1791 grams	22·1
B	100 +100 0·2 % HCl	0·1 % HCl	1·4897	1·5

As previously stated, pineapple juice, and the isolated ferment as as well, manifests its proteolytic action in an alkaline-reacting fluid, as well as in the presence of an acid or neutral reaction. When, however, the solution becomes strongly alkaline proteolytic action is quickly retarded, the ferment in this respect differing very decidedly from the related ferments papaïn and trypsin. Thus, the addition of small quantities of sodium carbonate to neutralized pineapple juice, a few hundredths of one per cent., produces no noticeable effect, but as the quantity is increased the retarding action of the alkali becomes more pronounced, until at last it checks the proteolytic action of the ferment altogether. This is clearly shown in the following experiments:

Experiment IX.—The acidity of the pineapple juice employed was equal to 0·462 per cent. HCl. Necessary to neutralize 100^{cc} of juice, 13·4^{cc} of 5·0 per cent. solution of Na_2CO_3. The 10 grams of moist albumin coagulum used in the digestions contained 1·3516 grams of dry albumin. The mixtures were warmed at 40° C. for 1½ hours.

	Pineapple juice.	Reaction.	Undissolved albumin.	Per cent. digested.
A	100^{cc}	¾ neutralized	0·9663 gram	28·6
B	100	neutralized	0·9465	30·0
C	100	0·025 % Na_2CO_3	0·9522	29·6
D	100	0·05 "	0·9735	28·0
E	100	0·10 "	0·9968	26·3

Experiment X.—The acidity of the pineapple juice employed was equal to 0·656 per cent. HCl. Necessary to neutralize 100^{cc}, 19·1^{cc} of 5·0 per cent. solution of Na_2CO_3. The amount of dry albumin equivalent to the 10 grams of moist coagulum used in the digestions was 1·3468 grams. The mixtures were warmed at 40° C. for 2 hours.

	Pineapple juice.	Reaction.	Undissolved albumin.	Per cent. digested.
A	100^{cc}	neutralized	1·0257 grams	23·9
B	100	0·1 % Na_2CO_3	1·0577	21·5
C	100	0·5 "	1·2263	9·0
D	100	1·0 "	1·3520	0

Hence, as is evident from the above experiments, the addition of sodium carbonate to neutralized pineapple juice to the extent of 0·5 per cent., almost completely stops the action of the ferment, while the presence of 1·0 per cent. of the alkali carbonate checks it altogether. Doubtless, the isolated ferment would show a still greater susceptibility to the action of dilute alkaline fluids.

From the foregoing, it is evident that digestion with bromelin, the ferment of pineapple juice, goes on most vigorously in neutral solutions, but that the presence of small amounts of acid, especially such as are contained in pineapple juice, and of sodium carbonate interfere with the proteolytic action only slightly; larger amounts, however, check the action of the ferment altogether.

It is further evident from the foregoing results that the proteolytic ferment of pineapple juice is an exceedingly vigorous ferment. We cannot say definitely how much pure ferment by weight is contained in 100^{cc} of filtered pineapple juice. There is no doubt that the amount varies greatly in different specimens of fruit; in fact, our results show plainly differences in proteolytic power hard to be accounted for in any other way. Experiments to be described

later show that the proteolytic ferment is either precipitable by heat, or else is associated with proteid bodies so precipitated. Now since the total amount of matter precipitable by boiling from 100^{cc} of filtered pineapple juice amounts to only 27 milligrams, and this obviously cannot be all proteolytic ferment, it is probable that the amount of pure ferment contained in the quantity of pineapple juice used in the various digestions recorded does not amount to more than a few milligrams, and yet in one experiment with the above quantity of ferment the equivalent of 1714 milligrams of dry muscle proteids were dissolved in one hour at 40° C., and of blood fibrin an amount equivalent to 1283 milligrams of dry proteid in two hours at 40° C.

With such vigorous digestive action as this, many possibilities suggest themselves in the way of practical application of the isolated ferment, or even of the pineapple juice itself. As a means of peptonizing foods it offers peculiar advantages in that the products of digestion, to be referred to later, are free from the objectionable taste usually associated with peptones resulting from the proteolytic action of animal ferments. Again, the ferment cannot but constitute a good solvent for pseudo-membranes, while its vegetable origin would perhaps recommend it as a more agreeable remedy than the kindred ferments from animal tissue. In some sections, popular opinion has already accredited to pineapple juice virtue as a solvent for the false membranes formed in diphtheria, a belief which is now seen to be founded on a reliable basis.

2.—*Influence of temperature.*

It is a matter of common observation that the digestive ferments, or enzymes, present in the animal organism act most energetically at approximately the body temperature, viz: 38°–40° C. Certain of the vegetable ferments on the other hand, notably the diastase of malt, act most vigorously at a higher temperature. With papaïn, the proteolytic ferment of papaw juice, Martin demonstrated the greater activity of the ferment at temperatures between 30° and 36° C. than between 18° and 20° C. in neutral solutions,* but apparently the effect of higher temperatures was not tried.

Experiment XI.—The 10 grams of moist albumin coagulum used in the digestions contained 1·4990 grams of dry albumin. The several mixtures were warmed with the albumin at the stated tem-

* Journal of Physiology, vol. v, p. 221.

peratures for 2¼ hours, the juice having been first brought to the desired temperature prior to the addition of the albumin.

	Neutralized pineapple juice.	Temperature.	Undissolved albumin	Per cent. digested.
A	100 c. c.	12° C.	1·3090 grams	12·7
B	100	20	1·3037	13·1
C	100	40	1·2281	18·1
D	100	49	1·1959	20·3
E	100	56	1·1709	21·9

Although in this experiment, the proteolytic action of the juice, for some reason, was not as great as usual the results show in a general way that the activity of the ferment increases with the rise in temperature up to 56° C. Further, that the ferment is active at comparatively low temperatures, although there is a striking difference (nearly 50 per cent.) in activity between the two extremes, viz: at 12° and 56° C. That this peculiar ferment is truly more active at 50°–60° C. than at 30°–40° C., under the above conditions, is confirmed by the two following experiments:

Experiment XII.—The weight of dry albumin equivalent to the 10 grams of moist coagulum used in each digestion was 1·2937 grams. The several portions of neutralized pineapple juice were brought to the required temperatures in carefully regulated water-baths, and when the desired point was reached *the albumin was at once added* and the mixtures kept at the stated temperatures for two hours, after which, as in the other experiments, the undissolved albumin was filtered off, washed, dried and weighed.

	Neutralized pineapple juice.	Temperature.	Undissolved albumin.	Per cent. digested.
A	100 c. c.	40° C.	1·0259 grams	20·7
B	100	49	0·9648	25·5
C	100	58	0·9337	27·8
D	100	66	0·9721	24·9

Experiment XIII.—This experiment was conducted in essentially the same manner as the preceding, but at different temperatures. The weight of dry albumin equivalent to the 10 grams of moist coagulum used in the individual digestions was 1·3710 grams. The ferment was allowed to act on the albumin for two hours at the respective temperatures. In this experiment, duplicate digestions were made and the results are interesting as showing about how much variation may be expected from the errors naturally incidental to methods of this character.

	Neutralized pineapple juice.	Temperature.	Undissolved albumin.	Per cent. digested.
A	100 c. c.	50° C.	0·9190 gram	33·0
B	100	60	0·9289	32·3
C	100	60	0·9286	32·3
D	100	70	1·0669	22·2
E	100	70	1·0562	23·0
F	100	80	1·3665	0·4

From these two experiments it is plain that the ferment as contained in *neutralized* pineapple juice is most active, on coagulated egg-albumin at least, between the temperatures of 50° and 60° C. and, further, that even at 70° C. the ferment is decidedly active. At 80° C. there is practically no action whatever. In this connection it is to be remembered that neutralized pineapple juice when subjected to heat precipitation grows slightly turbid at 72°–74° C., with separation of a flocky precipitate at about 82° C. Doubtless the destruction of the ferment by heat above 70° C. is associated with this precipitation, it being quite possible that it is the destroyed ferment itself which is so precipitated.

When pineapple juice of *natural acidity* is warmed with egg-albumin at the above high temperatures, a result quite different from the preceding is obtained; under such conditions, the proteolytic action of the ferment is diminished rather than increased and at 70° C. or under, the ferment is completely destroyed. This is shown in the following experiment.

Experiment XIV.—The pineapple juice employed had an acidity equal to 0·454 per cent. HCl. The 10 grams of moist coagulum used in the digestions contained 1·2956 grams of dry albumin. The mixtures of pineapple juice and albumin were warmed at the given temperatures for two hours, 100 c. c. of pineapple juice being used in each digestion.

Temperature.	Reaction.	Undissolved albumin.	Per cent. digested.
40° C.	natural acidity*	0·9667 gram	25·4
40	neutral	0·9679	25·3
55	natural acidity	1·0672	17·7
55	neutral	0·8968	30·8
70	natural acidity	1·3012	0
70	neutral	1·0581	18·4

From these results it is seen that while the neutralized fluid is more active at 55° C. than at 40° C., thus confirming the previous

* In this individual experiment, the neutralized and acid fluids, for some reason, show exactly the same digestive power at 40° C.

data, the acid-reacting fluid, on the other hand, shows far less digestive power at 55° C. than at 40° C. and further, at 70° C. is entirely devoid of digestive action, while the neutral fluid at the latter temperature is strongly proteolytic. The general trend of these results, therefore, is to show that the ferment in a neutral solution will withstand exposure to high temperatures better than in an acid-reacting fluid ; and further, that while the ferment in a neutral solution, as in neutralized pineapple juice, acts most energetically between 50° and 60° C., in an acid solution proteolytic action is most vigorous in the neighborhood of 40° C.

With pepsin, Biernacke* has shown that an acid solution increases the resistance of the enzyme to the destructive action of a high temperature ; thus this ferment in the presence of 0·2 per cent. hydrochloric acid may be heated up to 60° C. before it is killed, while in a neutral solution of the same strength the ferment is destroyed at 55° C. Trypsin, on the other hand, was found more resistant to heat in an alkaline fluid than in a neutral or weakly acid solution.

In this connection it is interesting to notice that the acid-reacting pineapple juice (natural acidity) subjected to heat precipitation grows turbid at 60°–62°, with separation of flocks at about 75° C.; in other words, the destruction of the ferment by heat in the acid-reacting fluid is coincident with the commencement of the precipitation, the same as in the neutralized juice.

While *neutralized* pineapple juice is extremely active on proteid matter at the high temperatures stated, exposure of the ferment solution by itself *in the absence of any proteid matter*, to the above temperatures for even a short time quickly destroys the ferment. This fact is clearly shown in the following experiment :

Experiment XV.—Given volumes of neutralized pineapple juice were placed in water-baths having the desired temperatures, and when the solutions themselves had reached the temperatures stated in the following table they were kept at that point for fifteen minutes, after which they were removed from the baths, cooled to 40° C. and 10 grams of coagulated egg-albumin added. The mixtures were then warmed at 40° C. for two hours and the proteolytic action determined in the usual manner. The amount of dry albumin (at 110° C.) equivalent to the 10 grams of moist coagulum used in the individual digestions was 1·3364 grams.

* Das Verhalten der Verdauungsenzyme bei Temperaturerhöhungen. Zeitschrift für Biologie, Band xxviii, p. 49.

	Neutralized pineapple juice.	Warmed for 15 minutes at	Undissolved albumin.	Per cent. digested.
A	100 c. c.	40° C.	1·1060 grams	17·3
B	100	60	1·2049	9·9
C	100	70	1·2971	3·0
D	100	80	1·3420	0

While neutralized pineapple juice is capable of digesting more proteid matter at 60° C. than under like conditions at 40° C., warming the ferment solution alone at 60° for fifteen minutes, prior to the introduction of the albumin, diminishes the proteolytic power of the ferment full 50 per cent. This result suggests that the products of digestion protect the ferment, to a certain extent, from the destructive action of the high temperature. The ferment acts quickly on proteid matter, so that even after a few minutes exposure of a mixture of albumin and pineapple juice to 40°–70° C., some proteoses and peptone are doubtless formed, which can in some manner exert their protective action. When, however, the neutralized juice alone is heated to 60° C., in the absence of proteoses and peptone, the ferment is rapidly destroyed. Doubtless, the ferment as contained in pineapple juice is more resistant to heat, than a solution of the isolated ferment would be, from the possible protective action of salts present, although of this point we cannot speak definitely. These results accord with Biernacki's* observations on the ferments pepsin, trypsin and ptyalin. This investigator found that albumose, amphopeptone and antipeptone raised the temperature at which trypsin was destroyed five degrees or more; that peptone raised the temperature at which pepsin was destroyed in acid solution, from 50°–55° C. up to 70° C. These results may perhaps be explained on the ground of a combination of the ferments with the albuminous bodies, the hypothetical compounds having greater resistance towards heat than the ferment alone. In any event, the general statement may be made that the purer the ferment the less resistant will it probably be to the destructive action of high temperatures.

While the preceding results show that the pineapple ferment, as contained in filtered pineapple juice, is liable to be killed on long-continued exposure to temperatures favorable for its proteolytic action, the fluid may be heated at temperatures under 40° C. for long periods of time, or even evaporated to dryness, without destruction of the ferment, provided the temperature is carefully regulated and

* Zeitschrift für Biologie, Band xxviii, p. 49.

not allowed to pass beyond 40° C. This is demonstrated by the following experiment:

Experiment XVI.—The pineapple juice employed had an acidity equal to 0·445 per cent. HCl, 100 c. c. requiring 13·0 c. c. of a 5·0 per cent. solution of sodium carbonate for neutralization. The 10 grams of albumin coagulum used in the digestions contained 1·3633 grams of dry albumin.

A	100 c. c.	pineapple	juice	+13·0 c. c. 5 per cent.	Na_2CO_3 sol.
B	100 "	"	+13·0 "	"	" "
C	100 "	"	+13·0 "		H_2O
D	100 "	"	+13·0 "		"

Solutions B and D were evaporated to dryness on plates at 40° C., the residues dissolved in water and made up to 113 c. c. respectively. All four solutions were then mixed with 10 grams of albumin coagulum and warmed at 40° C. for 3½ hours, after which the amount of albumin digested was determined in the usual manner.

	Reaction, etc.	Undissolved albumin.	Per cent. digested.
A	neutral	0·8177 gram	40·1
B	" evaporated	1·0215	25·1
C	natural acidity	0·8932	34·5
D	" evaporated	0·8638	36·7

From these results, it is plain that pineapple juice of natural acidity can be evaporated to dryness at a temperature not exceeding 40° C. and still preserve its proteolytic power. With neutralized juice, however, the above results indicate a partial destruction of the ferment during the evaporation. Whether this is due to the action of the neutral salts formed by neutralization of the acid, or to some other cause we cannot say. Possibly, the solution may have been made slightly alkaline, which would naturally give rise to some destruction of the ferment. Several repetitions of the above experiment, less carefully conducted, have shown that evaporation of the acid juice, however, is very liable to result in a partial loss of proteolytic power, unless great care is taken in keeping the evaporating fluid at 40° C. or under.

3.—*Rate of action.*

Pineapple juice is not only exceedingly active on proteid matter, but under favorable circumstances the digestive power of the ferment is quickly manifested. On blood fibrin and muscle tissue, especially, the proteolytic action of the ferment is shown in a *rapid*

solution of the proteid substance ; in fact, a single observation of the manner in which blood fibrin is attacked by pineapple juice is sufficient to give one a just appreciation of the energy of the ferment. Thus in Experiment IV, it will be remembered that 50 per cent. of the blood fibrin used in the experiment was converted into soluble products in two hours, and that in Experiment V, with the proteids of muscle tissue, 58 per cent. of the proteid matter was dissolved by neutral pineapple juice in half an hour at 40° C. Naturally, on coagulated egg-albumin the digestive power of the ferment is less quickly manifested, but the experiments already recorded show that even with this more difficultly digestible proteid, the rate of action is fairly rapid. The two experiments following give a general impression of the rate of action of the ferment, in the digestion of coagulated egg-albumin.

Experiment XVII.—The 10 grams of albumin coagulum used in the digestions contained 1·3944 grams of dry albumin. The mixtures were warmed at 45° C. for the periods stated in the following table, the amount of albumin digested during the periods being then determined in the usual manner.

	Neutralized pineapple juice.	Time.	Undissolved albumin.	Per cent. digested.
A	100 c. c.	½ hour	1·3026 grams	6·6
B	100	1	1·2072	13·5
C	100	2	1·0827	22·4
D	100	4	0·8814	36·8
E	100	5	0·8521	38·9

Experiment XVIII.—The 10 grams of albumin coagulum used in the digestions contained 1·4333 grams of dry albumin. The mixtures were warmed at 40° C. for the different periods stated, after which the undissolved albumin was filtered off, washed and weighed.

	Neutralized pineapple juice.	Time.	Undissolved albumin.	Per cent. digested.
A	100 c. c.	¼ hour	1·3201 grams	7·9
B	100	½	1·2472	13·0
C	100	1	1·1554	19·4
D	100	2	1·1366	20·7
E	100	4	1·0117	29·5
F	100	6	1·0012	30·2

While the results of these two experiments differ somewhat from each other in some respects, they are alike in showing that the ferment commences to act upon the proteid matter at once, and that this digestive action steadily continues, in the case of the above proteid, for about four hours, after which time the action becomes very much slower.

Separation of the proteolytic ferment.

Saturation of neutralized pineapple juice with ammonium sulphate precipitates, as already stated, all of the proteid matter contained in the solution. The filtrate does not give the slightest trace of a turbidity on boiling, even after the addition of acetic acid. Nitric acid likewise fails to give any reaction. Acetic acid and potassium ferrocyanide, however, give a slight flocky precipitate, which does not appear to be composed of proteid matter. The ammonium sulphate precipitate contains all of the proteolytic ferment and likewise the milk-curdling ferment, if this is a distinct body. It is readily and completely soluble in water, and by long continued dialysis the solution can be freed from ammonium sulphate. Unless every trace of adherent salt is removed from the solution, the fluid remains fairly clear, but the reaction so far as our experience extends becomes, almost invariably, slightly alkaline. On evaporation of the fluid, after removal of all, or nearly all of the salt, a scaly residue is obtained readily soluble in water, or in the slight trace of salt present, with strong proteolytic power and giving distinct reactions with the xanthoprotein, biuret and Millon's test. The aqueous solution is usually slightly turbid, the turbidity however disappearing on the addition of a little salt-solution, especially with the aid of a gentle heat, also on the addition of 0·2 per cent. hydrochloric acid. Evidently, the bodies composing the ammonium sulphate precipitate are readily soluble in very dilute salt-solution, if not in water alone.

This method constitutes a fairly good way of separating the ferments from the bulk of the extraneous matters present in the juice. We have not, however, spent much time in a close study of the make-up of this product as it is obviously a mixture of essentially all the proteid bodies contained in the juice, but it makes a very good preparation with which to demonstrate the proteolytic and milk-curdling properties of the ferments.

The most satisfactory method we have thus far found for the isolation of the proteolytic ferment, and one which yields a product with very strong digestive power, is by precipitation with common salt.

Saturation of neutralized pineapple juice with sodium chloride gives rise to a small flocculent precipitate, which is not at all increased by the addition of acetic acid to the salt-saturated fluid. Obviously, this precipitate might be composed of a globulin, or of a body akin to heteroproteose. It was studied after the following

plan : a large volume of freshly filtered juice was carefully neutralized with sodium carbonate, and then saturated at the temperature of the room with pure salt. The slight flocculent precipitate which resulted was filtered off, washed with saturated salt-solution, then dissolved in water and dialyzed in running water, putrefaction being prevented by addition of thymol. After several days' dialysis, though still containing some sodium chloride, a portion was tested as follows : it had the appearance of a somewhat turbid fluid, as though a separation had commenced to take place, but it could not be filtered clear. On warming the mixture gently, the turbidity showed a tendency to clear up somewhat. Subjected to careful heating, the mixture appeared to show an increased turbidity at 65° C., while at 72° C. there was a distinct and heavy turbity, but no separation of flocks. As the temperature was raised, the turbidity changed to a thick milkiness without, however, any appearance of flocks even on boiling. The solution seemed perfectly neutral to delicate test papers. By judicious addition of dilute acetic acid and renewed heating, the milky fluid was finally made to yield a flocky precipitate, in the filtrate from which no proteid reaction could be obtained, thus indicating that the sodium chloride precipitate is composed wholly of matter precipitable by heat.

The solution taken from the dialyzer showed strong proteolytic action on blood fibrin.

The remainder of the solution was dialyzed for one week longer, until nearly every trace of sodium chloride was removed, and again tested. The fluid had a very milky appearance, but no distinct separation of flocculent matter was seen. This heavy turbidity cleared up somewhat on warming and disappeared completely on adding a few drops of 20 per cent. salt-solution, also on the addition of a drop or two of dilute nitric acid. The addition of more nitric acid to the latter solution was followed by the reappearance of a turbidity, which did not disappear by gentle heat, but separated into flocks, not readily soluble in a large excess of nitric acid.

The reaction of the solution taken from the dialyzer was faintly alkaline. Heated, the solution became distinctly turbid at 65°–70° C. and quite opaque at 80° C., but without separation of flocks until after persistent boiling. A drop of acetic acid, however, added to the boiling fluid quickly caused a flocculent precipitate, the filtrate from which was practically free from all trace of proteids. The salt-free solution, although faintly alkaline, still showed strong proteolytic power.

On cautiously adding 0·2 per cent. hydrochloric acid to the faintly alkaline fluid a heavy turbidity made its appearance, which changed to a flocculent precipitate as the fluid became slightly acid; on addition of a small excess of acid the precipitate quickly dissolved. From this it may be inferred that if the solution had been perfectly neutral, a more pronounced separation of the proteid might have occurred in the dialyzer tube; evidently, however, the body is extremely soluble in very dilute acid and alkaline solutions, as well as in dilute solutions of neutral salts. Thus, the precipitate produced in this manner by the addition of a little 0·2 per cent. hydrochloric acid was readily dissolved by 10 per cent. salt-solution, the fluid becoming turbid again on boiling, with separation of a flocculent precipitate.

Further, by gently warming the turbid mixture resulting from the addition of a few drops of 0·2 per cent. hydrochloric acid, the fluid cleared up almostly completely, the turbidity returning as the mixture cooled.

Addition of dilute hydrochloric acid, however, did not precipitate all of the proteid; the filtrate still contained some proteid matter and, moreover, showed marked proteolytic action on fibrin. Thus in one instance, 0·2 per cent. hydrochloric acid was added to the dialyzed solution until a distinct flocky precipitate resulted, this was filtered off and dissolved in 10-per cent. solution of sodium chloride. This solution on being heated gradually, grew turbid at 66°–68° C. but did not yield any further precipitate even on boiling. The turbidity did not disappear on the addition of 0·5 per cent. sodium carbonate, or of 0·2 per cent. hydrochloric acid, but was soluble in dilute potassium hydroxide.

The filtrate from the above precipitate showed marked proteolytic action; it also yielded a slight precipitate with strong nitric acid, not dissolved by warming; subjected to heat precipitation the solution grew turbid at 73° C., but did not give any further precipitate even on boiling. The turbidity did not readily disappear on the addition of either dilute hydrochloric acid or of 0·5 per cent. sodium carbonate, but was quickly soluble in very dilute potassium hydroxide. Evidently, the substance precipitated by the acid and that remaining in solution were essentially the same.

From the foregoing, it is plain that the proteid substance precipitated by saturation of neutral pineapple juice with sodium chloride, is a peculiar body partaking both of the characters of a globulin and of heteroproteose. That it approximates more closely to the latter

is evident from the fact that the several precipitates produced by heat are more soluble in dilute alkalies than is customary for coagulated globulin, and that the precipitate produced by addition of dilute hydrochloric acid is so readily soluble on warming the neutral or slightly acid mixture, followed by its re-appearance as the temperature is lowered. On the other hand, the pronounced insolubility of the proteid in warm nitric acid of all strengths is contrary to the usual behavior of the proteoses. Further, the substance gives a biuret reaction more violet than red, and when boiled for a short time with 0·5 per cent. sodium carbonate it is transformed apparently into alkali-albumin, since the precipitate resulting from neutralization of the alkaline fluid is then insoluble in salt solution, although readily dissolved by a slight excess of dilute hydrochloric acid.

It is further evident that the proteolytic ferment of pineapple juice is either associated with this peculiar globulin or proteose-like body, or else is the body itself. The above method of precipitating the proteolytic ferment, by saturation of the juice with sodium chloride, brings about a complete separation of the ferment, provided the fluid is fully saturated with the salt. The filtrate, however, still contains some proteid matter, precipitable by shaking the fluid with neutral ammonium sulphate. This substance was separated from a large quantity of juice, by treating the filtrate from the sodium chloride precipitate with ammonium sulphate, added to complete saturation.

The slight stringy precipitate which resulted was filtered off, dissolved in water and dialyzed. After several days a small portion of the solution, though still containing some salts, was tested as follows: heated gradually the solution became slightly turbid at 65°–70° C., with separation of flocks at 80°–82° C. The filtrate from this precipitate gave no further signs of separation even when heated to boiling, but a drop of acetic acid added to the hot fluid produced a slight turbidity.

The dialysis was continued for about ten days longer, until the salts were almost wholly removed, when the solution was found faintly alkaline and slightly turbid. This turbidity disappeared at once on addition of a drop of dilute nitric acid, also on the addition of a few drops of salt solution. Addition of 0·2 per cent. hydrochloric acid to faint acid reaction produced a slight precipitate, readily soluble in salt solution. Subjected to heat precipitation, the turbid fluid cleared up somewhat at first, then became slightly turbid

at about 68° C., the turbidity becoming more pronounced at 75°–80° C., followed by the separation of a fine flocculent precipitate at 85°–87° C. A more complete separation was obtained by adding a drop of acetic acid to the hot fluid. The precipitate produced by heat at about 85° C. was almost wholly insoluble in dilute alkalies.

This ammonium sulphate precipitate was found wholly free from any proteolytic power either in neutral, acid or alkaline solutions. With the biuret test it gave a very faint violet color.

Hence, as already stated, saturation of neutral pineapple juice with sodium chloride precipitates all of the proteolytic ferment present, while there remains in solution some proteid substance, precipitable by ammonium sulphate, having essentially the same chemical properties as the preceding body. Certain points of difference, however, are noticeable; thus the body precipitated by sodium chloride shows more of a tendency to separate from its solution on dialysis, than the substance precipitated by ammonium sulphate. Further, the former body on exposure to heat precipitation separates less readily from its solution, except on the addition of a drop of acid, although the solution shows a heavy turbidity at much the same temperature as the latter body. The one thing, however, to be emphasized here is that the sodium chloride precipitate is strongly proteolytic, while the body separated by ammonium sulphate is devoid of this property. A more exact study of the chemical status of these two substances is now in progress, but at present nothing more definite can be said.

Another method of separating the proteolytic ferment is by precipitation with magnesium sulphate. Saturation of neutralized pineapple juice with this salt gives much the same separation as that produced by saturation with sodium chloride; i. e. a slight flocky precipitate, readily soluble in water and, after removal of the adherent salt, strongly proteolytic. The filtrate still contains some proteid matter, precipitable in small quantity by the addition of sodium sulphate in substance.

The magnesium sulphate precipitate was examined with the following results; it was dissolved in water and dialyzed for several days, until the greater portion of the adherent salt was removed. On testing the solution by heat precipitation, it grew distinctly turbid at 62° C., with separation of flocks at 75° C. This precipitate represented practically all of the proteid present in the solution, for boiling the filtrate, with or without acid, failed to give any further separation other than a faint turbidity produced by the

acetic acid. The dialysis was continued for a week or more longer, until every trace of sulphate was removed, when the reaction of the solution was found distinctly alkaline, so much so that no precipitate separated even on boiling the solution, although it became quite opaque at about 78° C., until a drop of acetic acid was added. In another preparation, where the alkalinity was less pronounced, the solution after dialysis of all, or nearly all of the salt, gave a flocculent precipitate at 85° C.

The faintly alkaline solution of the proteid yielded a precipitate on addition of 0·2 per cent. hydrochloric acid, very soluble in a little salt solution and in a slight excess of 0·2 per cent. acid. Only a portion of the substance was separated however, by this method of precipitation. The filtrate, which appeared nearly neutral to test papers, on being heated grew turbid at 73° C., without however any separation of flocks until the solution was boiled. The slight flocculent precipitate which then resulted was wholly soluble in 0·5 per cent. sodium carbonate, and nearly so in 0·2 per cent. hydrochloric acid. Further, the filtrate had marked proteolytic power and treated with nitric acid gave a distinct turbidity, not appreciably diminished by warming the mixture. That portion of the proteid precipitated by the dilute hydrochloric acid gave, after solution in dilute sodium chloride, essentially the same reactions as the foregoing, so that evidently the two fractions were identical.

This preparation of the ferment agreed fairly well in its reactions with the substance separated by sodium chloride. Like the latter, the turbid solution resulting from the dialysis cleared up on addition of a little salt solution, likewise on the addition of a few drops of dilute nitric acid, while more acid produced a second turbidity not readily dissolved by an excess of the acid, or by heat. With the biuret test, a more distinctly reddish color was produced than heretofore seen.

There are, therefore, two good methods for the isolation of the proteolytic ferment from pineapple juice; one by saturation of the neutralized fluid with sodium chloride, the other by saturation with magnesium sulphate. In our opinion, the former method yields a product with the strongest proteolytic power, although this needs to be verified by further observations. As to the exact chemical nature of the ferment, or of the body it is associated with, we are not yet able to speak with perfect confidence. It is our opinion that the proteolytic body separated by the above methods is a mixture of a globulin and a proteose, but as yet we have not been able to accomplish a dis-

tinct separation. On the other hand, it is possible that the substance is a single body possessed of properties akin to both the above, but this view seems hardly probable; its precipitation by saturation with magnesium sulphate and by sodium chloride possibly favor its being a globulin, while its extreme solubility in dilute salt solutions, in dilute acids and alkali are equally characteristic of both bodies. The more or less constant solubility of the heat precipitates in dilute alkalies favors its proteose nature, and if a proteose it is most closely related to heteroproteose. The substances are, moreover, alkaline reacting bodies completely precipitable by heat, especially in the presence of a trace of acid.

We are now occupied, with the aid of larger quantities of material, in an attempt at a better separation of these bodies with the hope of acquiring more definite knowledge regarding their chemical nature, composition, etc.

Whether globulin or proteose, these bodies present in pineapple juice, are very resistant to the digestive action of the ferment. Thus, long continued warming (3–5 hours) of fresh pineapple juice with strong proteolytic power at 40° C. does not, in the least, change the temperature at which the several heat precipitations occur; a point which certainly indicates the resistance of these proteids to proteolytic, or at least to this particular kind of proteolytic, action; the bodies in this respect resembling the *atmid* bodies described by Neumeister.*

Products formed by the proteolytic action of the ferment.

On this point, we have made only a few preliminary experiments, designed simply to throw some light on the nature of the ferment as a proteolytic agent. The results indicate that the ferment is more nearly related to trypsin than to pepsin, in that not only are proteoses and peptone formed by its action, but also leucin and tyrosin.

When washed and boiled blood fibrin, for example, is warmed at 40° C. with fresh pineapple juice of *natural acidity* for two or three hours, the proteid matter is thoroughly digested, but, as previously stated, a fairly large residue of finely divided matter remains undissolved, resistant to the further action of the ferment. This antialbumid-like matter is readily soluble in weak solutions of sodium carbonate, from which it is re-precipitated by addition of acetic acid,

* Zeitschrift für Biologie, Band xxvi, p. 57. "Ueber die nächste Einwirkung gespannter Wasserdämpfe auf Proteine und über eine Gruppe eigenthümlicher Erweisskörper und Albumosen."

and not readily dissolved by an excess of the acid. This same body is likewise formed by the action of fresh pineapple juice in neutral or weak alkaline solutions, and also by the isolated ferment as prepared by the methods previously described.

Boiling the acid digestive fluid gives no noticeable coagulum, and addition of moderately strong nitric acid likewise fails to produce any precipitate. Neutralization of the acid digestive mixture may give rise to a small amount of a neutralization precipitate, resembling acid-albumin. On evaporation of the neutralized fluid, the solution remains fairly clear, and when sufficiently concentrated addition of strong alcohol gives a gummy precipitate of proteose and peptone, while from the alcoholic solution crystals of tyrosin can be obtained and, in lesser quantity, leucin also. Tyrosin appears to be present in much larger amount than leucin. On dissolving the alcohol precipitate in water and saturating the solution with ammonium sulphate a heavy precipitate of proteose is obtained, while in the filtrate a large amount of true peptone is found, which after removal of the sulphate by dialysis gives a bright red color with the biuret test, etc. So far as our experiments extend, the proteose is composed mainly of a deutero-like body, only a small precipitate being obtained by saturation with sodium chloride, either in a neutral or slightly acid solution. An experiment like the preceding carried out with coagulated egg-albumin gave essentially the same results.

When pineapple juice of *neutral or faintly alkaline reaction*, or a *neutral or slightly alkaline solution* of the proteolytic ferment, is warmed with blood fibrin, for example, the digestion appears much the same as the preceding, but on heating the filtered solution a very pronounced milky turbidity appears, which on addition of a drop or two of acetic acid changes to a heavy flocculent precipitate, insoluble in excess of the acid.

Further, addition of nitric acid to the neutral or slightly alkaline digestive fluid gives a heavy curdy white precipitate, insoluble on application of heat, and likewise insoluble in an excess of the acid. Heated with an excess of acid, the precipitate takes on an intense yellow color. This precipitate produced by nitric acid is likewise insoluble in a 10-per cent. solution of sodium chloride, at least in the presence of the acid.

This body, so characteristic of bromelin digestion in *neutral or faintly alkaline* solutions, is very resistant to the action of the ferment, being still present even after a long-continued digestion. It seems probable that this substance results from the action of the fer-

ment in a neutral or alkaline solution, on the insoluble antialbumid-like body so conspicuous in an acid digestion; for while the latter body is also present in neutral and alkaline digestions, the amount seems smaller, and further, the reactions of the two bodies are very much alike.

Aside from this one point of difference, the products formed in a neutral or alkaline digestion are, so far as we have seen, essentially the same as those formed by pineapple juice of natural acidity. Thus, on boiling a slightly alkaline digestive mixture resulting from the action of the isolated ferment on blood fibrin, and adding a drop or two of acetic acid to facilitate the separation of the coagulum, a clear filtrate was obtained, without trace of reaction with nitric acid, except in the presence of salt, and giving the usual reactions for proteose and peptone. Thus, the addition of concentrated salt-solution to the clear filtrate, followed by a few drops of acid gave more or less of a turbidity, readily dissolved on application of heat, reappearing on cooling.

We hope soon to present a more detailed report regarding the properties and composition of the several products resulting from the action of the isolated ferment on egg-albumin, blood fibrin and myosin.

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